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AUTOLYSIS ON THE DIFFERENTIAL
SOLUBILITY OF THE PRINCIPLES
OF THE POSTERIOR LOBE OF
THE PITUITARY BODY

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

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EFFECT OF AUTOLYSIS ON THE DIFFERENTIAL SOLUBILITY OF THE PRINCIPLES OF THE POSTERIOR LOBE OF THE PITUITARY BODY^{1,2}

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THE ORIGINAL purpose of this investigation was to determine whether the marked disproportion between the pressor and the oxytocic principles in the posterior lobe of the pituitary glands of whales described by Geiling (1935) and associates (1936), was due to a differential autolysis of the principles of the gland before removal or to a disproportion peculiar to the species. Geiling and associates found the pressor activity of these glands to be 100 per cent of the standard, but the oxytocic content varied from 10 to 40 per cent of the standard. However, Oldham (1938) showed that a similar disproportion appeared in the armadillo under conditions that seem to exclude differential autolysis as the only explanation. In the latter work the glands were removed from the armadillos immediately after death. On the other hand, glands removed from the small white whale (porpoise) assayed 100 per cent of the standard for both hormones (Geiling and Coon). Therefore, the question still remained whether the previously reported departure from the 1:1 ratio between the pressor and the oxytocic hormones of the hypophysis of the large whales, which could only be removed from 10 to 24 hours after death, could be ascribed to autolysis, to species difference, or to both. However, numerous difficulties were encountered during the progress of this work, and the original purpose was abandoned in favor of the intensive study on the effect of solvents on powders prepared from the posterior lobes of autolyzed glands.

Numerous studies have been made on the effect of solvents on the hormones from the neural lobe of the hypophysis. Only those pertinent to this study are referred to here. Baudoin (1913) reported that cold alcohol was a poor solvent, but that boiling alcohol would dissolve the active principles. Crawford (1920) found that the pressor principle was insoluble in absolute alcohol at 20° C., but soluble in acidified alcohol. Kamm (1926) and his co-workers showed that acetone would dissolve the oxytocic principle four

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² The essential portions of this dissertation were presented before the American Society for Pharmacology and Experimental Therapeutics at the meetings in Baltimore, Maryland, 1938, and in Toronto, Canada, 1939.

times as readily as the pressor principle from the glands left for some hours at room temperature. They claimed that this increased solubility was due to the acidity produced by the glands on standing. In the work reported here, a study was made of the effect of varying periods of gland incubation on the differential solubility of the pressor and oxytocic principles in mixtures of warm solvents.

EXPERIMENTAL PROCEDURES

Three types of material were incubated, a) beef heads with the pituitary gland *in situ*, b) individual glands and c) posterior pituitary powder with and without fresh anterior and posterior lobe tissue or tissue juices added. These were incubated at 22 or at 37° C., for periods ranging from 1 to 48 hours.

a) Beef heads were incubated from 6 to 48 hours at 37° C., to simulate as far as possible the conditions of whale pituitary gland before its removal. The blubber of the whale is an effective insulating material, maintaining the temperature of the body while in the water for 10 hours or more after death. Forty-two beef heads in 11 different lots were obtained directly from the killing floor of an abattoir. They were taken to the laboratory within one hour after the death of the animals and incubated in a constant temperature room for varying lengths of time. After incubation, the pituitaries were dissected out and frozen with carbon dioxide snow. The posterior lobes were then separated and placed in C.P. acetone and made into powders from which solutions were prepared according to the directions in the U.S.P. XI. Glands in heads incubated 48 hours were too decomposed to permit differentiation of the lobes and could not be used.

b) The difficulties encountered in incubating whole beef heads led to a study under similar conditions of freshly removed beef pituitary glands. The moisture content of the glands was maintained by placing them in suitable containers. After incubation they were frozen with carbon dioxide snow, the lobes separated and placed in acetone to obtain a powder.

c) A series of experiments were carried out with 5 to 10 cc. volumes of 0.25 per cent acetic acid extracts of U.S.P. posterior lobe powder by incubating these extracts with fresh anterior or posterior lobe tissue or juices. These juices were prepared by macerating one gland in 5 cc. of saline. Two drops of this tissue extract were used for each experiment.

Quantities of 100 mg. of the different powders so prepared were extracted with acetone, methyl,³ ethyl, propyl, isopropyl, butyl, amyl and isoamyl alcohols by means of the micro-soxhlet extractor. Mixtures of acetone with the various alcohols were also used. The extractor held 10 cc. of solvent before siphoning over and the flask contained 20 to 22 cc. of solvent, depending on the relative quantities of the solvents present. When using mixtures of acetone and alcohol the amount of powder was kept constant so that the relative quantities of these two solvents condensed in the extractor would be uniform. The apparatus was set on a thermostatically controlled hot plate at

³ At the time this work was done the author was not aware of British Patent No. 334,898, which advocates the use of acidified methyl alcohol for extraction of the oxytocic principle.

60° C. Anhydrous materials were used and anhydrous conditions were maintained throughout the experiments by means of calcium chloride. The last condition was essential, as extraction over prolonged periods of time permitted the extracting menstruum to absorb enough moisture to destroy the activity of solute and residue.

The various extracts of the posterior lobe reported in this paper were assayed for both pressor and oxytotic potencies. The pressor activity was measured by the manometric blood pressure method on cats under sodium phenobarbital anesthesia. The oxytotic activity of the extracts was assayed by the chicken method recently described by Coon (1939). In most cases the results of the latter were verified by the official (U.S.P. XI) guinea pig uterus method.

RESULTS

Most of the powders made from glands incubated at 37° C. in the intact beef head for 24 hours or less, assayed 90 to 100 per cent of the standard. Such powders, with occasional exception, contained equal quantities of the oxytotic and pressor principles. The hormone content from the 11 different lots is shown in table 1.

TABLE 1. HORMONAL CONTENT OF POSTERIOR LOBES OF PITUITARY GLANDS INCUBATED AT 37° C. IN THE INTACT BEEF HEAD

NUMBER OF HEADS	HOURS OF INCUBATION	PERCENTAGE OF U.S.P. STANDARD	
		PRESSOR	OXYTOMIC
4	6	95-100	95-100
4	12	95-100	95-100
6	18	95-100	95-100
2	20	90-95	90-95
4	20	90-95	60-65
4	24	95-100	95-100
10	24	90-95	90-95
2	24	70-75	50-55
2	24	20-25	15-20
2	24	10 ¹	15-20
2	48	¹ Too decomposed to assay	

In a few instances these powders contained depressor properties. Abel (1919; 1920) and his associates, Dudley (1923), Dale and Dudley (1921), Jackson and Mills (1919), Hanke and Koessler (1920) and Schlapp (1925) have observed this depressor effect and have demonstrated that it is due to histamine and not to the active principles of the pituitary gland. When the powder from the glands incubated for 12 hours or more was extracted in a microsoxhlet extractor for 30 minutes with absolute ethyl alcohol to remove the depressor property the oxytotic activity of the extracted powder was found to be only 50 to 60 per cent of the standard, while the pressor activity remained unchanged. The remainder of the oxytotic activity was in the alcoholic extract. The glands from heads that were incubated for less than 12 hours did not show this separation of the oxytotic fraction so markedly.

In the series of experiments in which freshly removed glands with their

membranes intact were incubated in covered glass containers, essentially the same results were obtained as with glands incubated *in situ*. Glands incubated at room temperature gave more consistent results than those incubated at 37° C. Tables 2 and 3 give the data for this group of experiments.

The results of the experiments with the U.S.P. powder incubated with fresh anterior or posterior lobe tissue or juices were variable. In the majority of cases the loss of the activity was slight. In 30 such experiments inactiva-

TABLE 2. WHOLE PITUITARY GLANDS INCUBATED AT 37° TO 38° C.

NUMBER OF EXPERIMENTS	HOURS OF INCUBATION	PERCENTAGE OF U.S.P. STANDARD, XI	
		PRESSOR	OXYTOCIC
I	I	100	100
I	2	100	100
I	4	95-100	95-100
2	6	95-100	95-100
I	12	95-100	95-100
I	20	90-95	90-95
I	20	90-95	55-60
5	24	90-100	90-100
I	24	85-90	85-90
I	24	50	50
I	24	20-30	10-20
I	24	20-30	20-30

tion was observed in 8, in which the inactivation ranged from 10 to 90 per cent. The destructive effect of the fresh posterior lobe and anterior lobe tissues and tissue juices was essentially the same. This irregularity is not unique, since Kamm (*et al.*, 1926) found similar occasional losses in activity in powders prepared by the official method. At the present time no adequate explanation

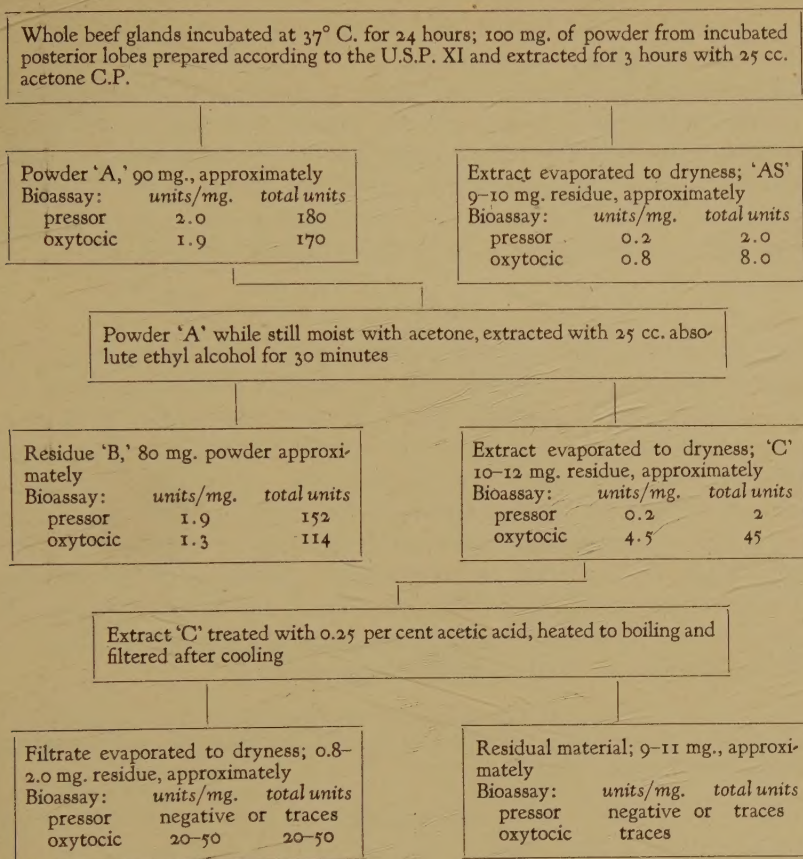
TABLE 3. WHOLE PITUITARY GLANDS INCUBATED AT 20° TO 22° C.

NUMBER OF EXPERIMENTS	HOURS OF INCUBATION	PERCENTAGE OF U.S.P. STANDARD	
		PRESSOR	OXYTOCIC
I	I	100	100
I	2	100	100
I	4	100	100
2	20	95-100	95-100
2	24	95-100	95-100
I	24	95-100	60-65

can be given for this occasional decrease in potency of the active principles and for the variations in the ratio between them. Heller (1937) has shown that adsorption may be a factor.

In using the various solvents it was noted that there was greater solubility of the active principles of the powder prepared from incubated glands than those of the powder prepared from fresh glands. In the extraction with hot acetone the hormones of the incubated powder showed greater solubility than did those of the standard powder. The oxytocic hormone was removed about four times as readily as the pressor hormone.

The flow sheet indicates the manner in which the various glands were processed, and the bioassay figures for one typical experiment.



Powders prepared from fresh pituitary glands obtained from the abattoir did not yield an alcohol-soluble oxytotic fraction after extraction for 30 minutes with absolute alcohol, as did powder 'A' from the incubated glands. According to Kamm and his collaborators when U.S.P. posterior pituitary powder is extracted in a soxhlet apparatus for 6 hours with absolute ethyl alcohol, approximately 10 per cent of the oxytotic hormone is removed. However, with autolyzed powder, in our experiments, extraction for as long as 70 to 100 hours with a 1:10 ratio of acetone to methyl alcohol gave a residual powder assaying 85 per cent pressor and approximately 5 per cent oxytotic activity. This is the most significant finding of this investigation.

A similar experiment was performed with sperm whale powder prepared by the official method and originally assaying 100 per cent pressor and 10 per cent oxytotic activity. In this case, soxhlet extraction was carried out for 65 hours with a 1:20 ratio of acetone to ethyl alcohol, and gave a residual powder containing 20 per cent pressor activity and no oxytotic activity.

The oxytocic hormone was more readily soluble in hot methyl alcohol than in hot ethyl alcohol. The greater losses in activity obtained on prolonged extraction with ethyl alcohol were no doubt due to the higher boiling point of this solvent. The other alcohols mentioned were found unsuitable because of their higher boiling points.

The foregoing experiments would seem, therefore, to comprise additional data on the differential solubilities of the pressor and oxytocic principles of the posterior pituitary. The facts presented, notably the greater solubility of the two fractions when derived from autolyzed glands, point rather to the prior existence of a 'mother substance' postulated by Abel (1930). This phase of the problem cannot, however, be further pursued here.

SUMMARY

The experimental data on beef pituitary glands incubated at 37° C. for twenty-four hours do not support the conception that the relatively low oxytocic content in the pituitary glands of the great whales is caused by autolysis. Many factors influence the loss of activity of the hormones; namely, enzymes, hydrogen ion concentration, adsorption, incubation time and temperature. A method for the separation of the pressor and the oxytocic hormones has been demonstrated by the use of mixtures of acetone with methyl or ethyl alcohol. The fact that the hormones in powders made from autolyzed glands are much more soluble in absolute alcohol and acetone than the hormones in powders made from unautolyzed glands suggest the possibility that these hormones may be derived from a larger molecule.

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